lines 1 and 2; page 4, lines 8-18 and page 7, lines 17-28; see also Example 2 where the production of marker-free transgenic plants is summarized in Table 10 on page 114 (see events MON850 in range 18, row 129, stake B and MON849 in range 20, row 102, stake A).

Support for marker genes, e.g. selectable or reporter genes (claim 53) and specific marker genes, e.g. *bar, nptII,* and glyphosate resistance genes (claim 54) can be found in the originally-filed specification at page 24, lines 11-22. New claim 55 characterized by a *uidA* gene, a *gfp* gene or an R-locus gene is supported in the originally-filed specification, at page 26, lines 26 to 30 and originally-filed claim 7.

Support for monocots (claims 56), specific monocots (claim 57), dicots (claim 58) and specific dicots (claim 59) is found in originally-filed claims 16, 17, 19 and 20, respectively.

Support for maize progeny produced by self pollination (claim 60), hybrid maize progeny produced by outcrossing (claim 61) and maize progeny produced by inbreeding (claim 62) is found in originally-filed claims 13, 14 and 15, respectively.

Support for frequency of deletion of at least 0.1% (claim 63), 0.6% (claim 64) and 2% (claim 65) is found in the originally-filed specification in Table 13 on page 116.

A clean copy of the new claims is submitted with this response in Appendix A.

A. Status of the Claims

Prior claims 2, 8-21 and 28-29 were elected in response to a restriction requirement dated June 5, 2002. After that election, the prosecution of parent patent application 09/521,557 resulted in allowed claims (see Appendix B). The requested amendment serves to present claims of differentiated scope from those allowed in the parent application.

B. Objection to the Specification

The specification is objected to for inclusion of an active hyperlink on page 32, line 16. Inspection of the application as filed indicates that page 32, line 26 reads "Adapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html." Enclosed is a substitute page 32 where the reference to the active hyperlink is replaced with the attribution "Adapted from Neil Crickmore, University of Sussex, UK." It is believed that this objection is obviated by this amendment. Attached as Appendix C is a substitute page 32 and a marked-up copy.

C. Obviousness-Type Double Patenting Rejection

Prior claims 2, 8-21 and 28-29 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 2-10 of

copending Application No. 09/521,557 (Appendix B). Applicant submits that the outstanding obviousness-type double patenting rejection is obviated by cancellation of the rejected claims.

Moreover, applicant provisionally traverses any further obviousness-type double patenting rejection of new claims 52-65 over claims of parent application Serial Application No. 09/521,557. Any such rejection must be based on an analysis analogous to a 35 U.S.C. 103 analysis. Such an analysis requires determining the scope and content of the claims of each application, ascertaining the differences between the claims of each application, resolving the level of ordinary skill in the art, and evaluating evidence of secondary considerations.

Applicant submits that a reasoned analysis of the claims will show significant differences. Simply stated, the allowed claims are directed to a method of preparing a fertile transgenic cereal plant having an altered transgene insertion while the instant claims are directed to a method of preparing a marker-free, fertile transgenic plant where a the marker gene is deleted. The claims are distinct, e.g. the instant claims could be infringed by applying the method to a dicot plant without infringing the claims in the parent application. In addition the allowed claims could be infringed by addition of a transgene without infringing the instant claims. If the rejection is to be maintained the PTO is requested to explain the motivation to a person of ordinary skill in the art to apply the method to plants other than cereal plants. Alleged obviousness for obviousness-type double patenting must be support by clear evidence. See *In re Kaplan* 229 USPQ 678 (Fed. Cir. 1986). Absent such motivation Applicant submits that such a rejection if maintained would be improper.

D. Rejection Under 35 U.S.C. §112, First Paragraph – Written Description

The rejection of prior claims 2, 8-21 and 28-29 under 35 U.S.C. 112, first paragraph, on the basis of an alleged failure of the specification to enable a transgene alteration other than deletion has been obviated by the cancellation of those claims. Regardless, applicant traverses this alleged failure and submits that a similar rejection has been rebutted in the parent application. In the event that the PTO would consider extending such a rejection to the new claims 52-65, applicant points out that the new claims are actually limited to a method of transgene deletion.

E. Rejection Under 35 U.S.C. §112, Second Paragraph – Indefiniteness

The rejection of prior claim 8 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter, e.g. for reciting "the

progeny transgenic plant cell", is obviated by the cancellation of claim 8. Should the PTO consider extending this rejection to the new claims, applicant points our that new claims 52-65 are not characterized by reference to "plant cells."

F. First Rejection Under 35 U.S.C. §102(b)

The rejection of prior claims 2, 8-15, 19, 21 and 28 as anticipated by Conner *et al.*, Molecular Breeding, 4:47-58, 1998 (Conner *et al.*) is obviated by the cancellation of those claims. Should the PTO consider extending this rejection to new claims 52-65 applicant offers the following observations explaining how Conner *et al* fails to teach or suggest the claimed method.

Inspection of Figure 1 in Conner *et al.* (page 49, column 1) shows that the NPTII selective marker gene alone is not flanked by any directly repeated sequences, rather the marker gene is flanked by a nosP promoter and either a Lhca3.ST.1 promoter or a dCaMV promoter. Thus, there were no directly repeated sequences surrounding the NPTII marker gene to allow for the deletion of the marker gene as claimed by a method of the practiced in the instant invention. A GUS selective marker gene is flanked by directly repeated nosT sequences. However, Conner *et al.* do not report any kanamycin resistant/GUS negative plants which would have resulted from a deletion of the GUS gene surrounded by direct repeats of the nosT sequence.

Conner *et al.* produced transgenic plants with directly-repeated MAR regions flanking a segment comprising BOTH the GUS and the NPTII marker genes. If homologous recombination between the MAR regions was responsible for the deletion of the GUS and NPTII genes, then progeny plants would have been NPTII sensitive and lacking GUS expression. But Conner *et al.* report a predominant deletion of the NPTII gene while retaining GUS expression which led Conner *et al.* to dismiss the possibility of homologous recombination by declaring in the paragraph bridging pages 55 and 56:

> "All 646 revertants observed among the self-pollinated progeny, and 49 revertants observed in the backcrossed progeny, were GUS positive. Therefore, they are unlikely to have resulted from the loss of transgenic sequences via homologous recombination."

Reference is also directed to the section in Conner *et al.* titled "*Concurrent loss of NPTII and GUS gene expression by recombination?*" where the high frequency of NPTII silencing is pondered. Despite a speculation that the "few seedlings identified as kanamycin-sensitive and

GUS-negative could have resulted from recombination events", Conner *et al.* teach away from the possibility of homologous recombination by concluding (page 56, end of first paragraph):

> "Such instabilities may represent a genomic flux resulting in the stochastic loss of genes or their expression rather than any specific genomic response to the presence of a transgene sequence."

Applicants also note that no molecular analysis was carried out on the plants showing kanamycin sensitivity or lack of GUS expression. Thus, Conner *et al.* cannot teach or suggest whether the changes in gene expression are due to deletion events or are the result of gene silencing mechanisms. In fact, Conner *et al.* admit that "PCR analysis proved highly unreliable in determining whether or not the NPTII gene was still present" (page 55, column 2, middle paragraph) and they further go on to state that "the precise reason for the loss of kanamycin resistance could not be definitively established" (page 55, column 2, middle paragraph). Conner *et al.* admit that revertants isolated from both heterozygous and homozygous transformants that were kanamycin sensitive were GUS positive, "therefore, they are unlikely to have resulted from the loss of transgenic sequences via homologous recombination" (page 55, column 2, last line to top of page 56, column 1, first paragraph).

Applicants submit that the Connor *et al.* reference teaches a person of ordinary skill in the art that the loss of gene activity is not the result of homologous recombination and as a result fails to suggest the method of applicants' invention applied to homozygous plants.

G. Second Rejection Under 35 U.S.C. §102(b)

The rejection of claims 2, 8-13, 15, 19, 21 and 28 under 35 U.S.C. 102(b) being anticipated by Assaad and Signer, *Genetics,* 132:553-566, 1992 (Assaad *et al.*) is obviated by the cancellation of those claims. Should the PTO consider extending this rejection to new claims 52-65 applicant offers the following observations explaining how Assaad *et al* fails to teach or suggest the claimed method.

The PTO has stated that the Assaad *et al.* reference teaches transformed *Arabidopsis* with a hygromycin gene flanked by two sections of an NPTII gene, deletion of which results in hygromycin sensitivity and kanamycin resistance upon restoration of the NPTII gene. Applicant argues that the post-recombination *Arabidopsis* plants described by Assaad *et al.*, while having lost the hygromycin gene, retain an NPTII marker gene. Applicants submit that Assaad *et al.* does not teach or suggest a method of producing a marker-free, fertile, transgenic plant.

H. Third Rejection Under 35 U.S.C. §102(b)

The rejection of claims 2, 8-13, 15, 19, 21 and 28 under 35 U.S.C. 102(b) being anticipated by Swoboda *et al.*, EMBO J, 13(2):484-489 (Swoboda *et al.)* is obviated by the cancellation of those claims. Should the PTO consider extending this rejection to new claims 52-65 applicant offers the following observations explaining how Swoboda *et al* fails to teach or suggest the claimed method.

The PTO has summarized that the Swoboda *et al.* reference teaches transformed *Arabidopsis* plants with a hygromycin gene flanked by two sections of GUS marker gene, deletion of which results in hygromycin sensitivity and GUS activity upon restoration of the GUS gene. Applicants submit that Swoboda *et al.* does not teach or suggest a method of producing a marker-free, fertile, transgenic plant.

I. Fourth Rejection Under 35 U.S.C. §102(b)

The rejection of claims 2, 8-11, 13-15, 19, 21 and 28 under 35 U.S.C. 102(b) being anticipated by Odell *et al.* in U.S. Patent 5,658,772 (Odell *et al.*) is obviated by the cancellation of those claims. Should the PTO consider extending this rejection to new claims 52-65 applicant offers the following observations explaining how Odell *et al* fails to teach or suggest the claimed method.

Odell *et al.* teaches a method for preparing transgenic tobacco plants having a deleted selectable marker where the marker was flanked by directly repeating lox sites and deletion was mediated by Cre recombinase. New claim 52 is directed to methods for deleting transgene marker flanked by directly repeated DNA sequences "wherein said directly repeated sequences are not recognized by a site-specific recombinase enzyme." Applicants submit that Odell *et al.* does not teach or suggest the claimed method of producing a marker-free, fertile, transgenic plant.

J. Fifth Rejection Under 35 U.S.C. §102(b)

The rejection of claims 2, 8-12, 14, 19, 21 and 28 under 35 U.S.C. 102(b) being anticipated by PURDUE WO 97/13401 (PURDUE) is obviated by the cancellation of those claims. Should the PTO consider extending this rejection to new claims 52-65 applicant offers the following observations explaining how Purdue fails to teach or suggest the claimed method.

PURDUE teaches a method for preparing transgenic *Arabidopsis* plants having a deleted selectable marker where the marker was flanked by directly repeating FRT sites and deletion was

mediated by FLP recombinase. New claim 52 is directed to methods for deleting transgene marker flanked by directly repeated DNA sequences "wherein said directly repeated sequences are not recognized by a site-specific recombinase enzyme." Applicants submit that PURDUE does not teach or suggest the claimed method of producing a marker-free, fertile, transgenic plant.

K. First Rejection Under 35 U.S.C. §103(a)

The rejection of claims 2, 8-18, and 21 under 35 U.S.C. 103(a) as being unpatentable over Odell *et al.* taken with D'Halluin *et al.* is obviated by the cancellation of those claims. As stated above Odell *et al.* fails to teach or suggest to a person of ordinary skill in the art a method of transgenic marker deletion without recombinase. Odell *et al.* teaches away from the use of directly repeated DNA sequences to effect alteration of a transgenic insertion without the use of site specific recombinase. Applicants submits that the PTO could not have made a *prima facie* case for obviousness for the new claims. New claim 52 is characterized by directly repeated DNA sequences "wherein said directly repeated sequences are not recognized by a site-specific recombinase enzyme." The primary reference Odell *et al.* does not teach or suggest recombination using directly repeated DNA sequences that are not recognized by a site-specific recombinase.

D'Halluin *et al.*, which is cited because it reports transformation of regenerable maize, does not cure this defect of Odell *et al.* in failing to teach or suggest the use of directly repeated DNA sequences or to practice gene alterations in the absence of a site-specific recombinase system.

What would motivate a person of ordinary skill in the art to discard both the lox recombination sites and the cognate recombinase used by Odell *et al.* for directly-repeating DNA sequences without a recombinase? What would be the motivation when Odell *et al.* declares that recombination does not occur without the recombinase? Applicants respectfully submit that that there is no support for a *prima facie* obviousness rejection of new claims 52-65 over Odell *et al* in light of D'Halluin *et al.*

L. Second Rejection Under 35 U.S.C. §103(a)

The rejection of claims 2, 8-18, and 21 under 35 U.S.C. 103(a) as being unpatentable over PURDUE taken with D'Halluin *et al.* is obviated by the cancellation of those claims. As stated above PURDUE fails to teach or suggest to a person of ordinary skill in the art a method

of transgenic marker deletion without recombinase. PURDUE teaches away from the use of directly repeated DNA sequences to effect alteration of a transgenic insertion without the use of site specific recombinase. Applicants submits that the PTO could not have made a *prima facie* case for obviousness for the new claims. New claim 52 is characterized by directly repeated DNA sequences "wherein said directly repeated sequences are not recognized by a site-specific recombinase enzyme." The primary reference PURDUE does not teach or suggest recombination using directly repeated DNA sequences that are not recognized by a site-specific recombinase.

D'Halluin *et al.*, which is cited because it reports transformation of regenerable maize, does not cure this defect of Purdue in failing to teach or suggest the use of directly repeated DNA sequences or to practice gene alterations in the absence of a site-specific recombinase system.

What would motivate a person of ordinary skill in the art to discard both the FLP recombination sites and the cognate recombinase used by PURDUE for directly-repeating DNA sequences without a *FLP* recombinase? Applicants respectfully submit that that there is no support for a *prima facie* obviousness rejection of new claims 52-65 over PURDUE in light of D'Halluin *et al.*

M. Third Rejection Under 35 U.S.C. §103(a)

The rejection of claims 2, 8-15, 19-21 and 28-29 under 35 U.S.C. 103(a) as being unpatentable over Odell *et al.* taken with Hinchee *et al.* is obviated by the cancellation of those claims. As stated above Odell *et al.* fails to teach or suggest to a person of ordinary skill in the art a method of transgenic marker deletion without recombinase and does not support a *prima facie* case for obviousness.

Hinchee *et al.*, which is cited because it reports transformation of soybean, does not cure the defects of Odell *et al.* in failing to teach or suggest the use of directly repeated DNA sequences or to practice gene alterations in the absence of a site-specific recombinase system.

What would motivate a person of ordinary skill in the art to discard both the lox recombination sites and the cognate recombinase used by Odell *et al.* for directly-repeating DNA sequences without a recombinase? What would be the motivation when Odell *et al.* declares that recombination does not occur without the recombinase? Applicants respectfully submit that that there is no support for a *prima facie* obviousness rejection of new claims 52-65 over Odell *et al.* in light of Hinchee *et al.*

N. **Fourth Rejection Under 35 U.S.C. §103(a)**

The rejection of claims 2, 8-15, 19, 21 and 28 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 5,876,988 (Selten *et al.)* taken with Lee *et al.*, Plant Cell, 2:415-425, 1990 (Lee *et al.*) is obviated by the cancellation of those claims. Selten *et al.* teaches the use of fungal or yeast transformation with a deletion of an acetamidase marker gene flanked by directly repeating sequences, after having grown putative transformants on a specific toxic chemical to effect deletion of a sequence which allows resistance to the chemical and thus cell survival. Selten *et al.* suggests the use of the technique in higher plants but, as recognized by the PTO, do not enable this technology in transformed plants.

Applicants note that new claim 52 is drawn to "obtaining a first fertile transgenic plant homozygous for a transgene insertion DNA sequence." Not only is the use of acetamidase not enabled as a marker for plants, but Selten *et al.* does not teach deleting markers from fertile, transgenic plants and Selten *et al.* does not suggest obtaining transgenic plants which are homozygous for a transgene insertion. It is well known that fungi, such as *Aspergillus nidulans* exist in a haploid state except briefly prior to meiosis; Selten *et al.* does not teach the preparation of homozygous microorganisms in the practice of the invention. How would a person of ordinary skill in the art contemplate creating the homozygotes specified in applicants' claimed method?

Lee *et al.* does not supplement the deficiency of Selten *et al.* in failing to teach or suggest the use of acetamidase as a plant marker let alone any method of transgene deletion in plants requiring obtaining homozygotes. Lee *et al.* teaches homologous recombination between an endogenous host DNA sequence and a transgenic DNA sequence introduced into tobacco via *Agrobacterium*. In addition, recombination using the method of Lee *et al.* results in plants which retain a marker gene in the organism. Applicant argues that the lack of marker-free plants taught by Lee *et al.* and the reliance of Selten *et al.* on acetamidase selection of microorganisms does not teach the instant invention directed to the preparation of marker-free plants selected from a homozygous population.

O. **Fifth Rejection Under 35 U.S.C. §103(a)**

The rejection of claims 2, 8-15, 19-21 and 28-29 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 5,876,988 (Selten *et al.)* taken with Lee *et al.* and Hinchee *et al.* is obviated by the cancellation of those claims. As discussed above, Selten *et al.* taken with Lee *et*

al. does not suggest the methods of new claims 52-65. Hinchee *et al.* adds nothing to the disclosures of Selten *et al.* and Lee *et al.* that would suggest the method of new claims 52-65 to a person of ordinary skill in the art.

P. Sixth Rejection Under 35 U.S.C. §103(a)

The rejection of claims 2, 8-15, 19-21 and 28 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 5,876,988 (Selten *et al.)* taken with Lee *et al.* and D'Halluin *et al.* is obviated by the cancellation of those claims. As discussed above, Selten *et al.* taken with Lee *et al.* does not suggest the methods of new claims 52-65. D'Halluin *et al.* adds nothing to the disclosures of Selten *et al.* and Lee *et al.* that would suggest the method of new claims 52-65 to a person of ordinary skill in the art.

Applicants submit that new claims 52-65 are patentable over the art of record as discussed in this response. An early favorable action with allowance of these claims is earnestly solicited.

Respectfully submitted,

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APPENDIX A; New claims

WHAT IS CLAIMED IS:

52. A method of preparing a marker-free fertile transgenic plant comprising:

a) obtaining a first fertile transgenic plant homozygous for a transgene insertion DNA sequence, wherein the transgene insertion DNA sequence comprises a marker gene DNA sequence flanked by directly repeated DNA sequences, wherein said directly repeated sequences are not recognized by a site-specific recombinase enzyme;

b) obtaining a plurality of progeny of any generation of the first fertile transgenic plant; and

c) selecting a progeny fertile transgenic plant wherein the marker gene is deleted.

53. The method of claim 52 wherein the marker gene comprises a selectable or reporter gene.

54. The method of claim 53 wherein the selectable marker gene comprises a *bar, nptII,* or a glyphosate resistant EPSPS enzyme gene.

55. The method of claim 53 wherein the reporter gene comprises a *uid*A gene, a *gfp* gene or an R-locus gene.

56. The method of claim 52 wherein the plant is a monocot.

57. The method of claim 56 wherein the monocot plant is a maize, barley, sorghum, wheat, rye or rice plant.

58. The method of claim 52 wherein the plant is a dicot.

59. The method of claim 58 wherein the dicot plant is a soybean, cotton, canola, or potato plant.

60. The method of claim 52 wherein said plant is maize and the plurality of progeny plants are obtained by self pollination.

61. The method of claim 52 wherein said plant is maize and the plurality of progeny plants are obtained by outcrossing to produce hybrid progeny.

62. The method of claim 52 wherein said plant is maize and the plurality of progeny plants are obtained by inbreeding to produce inbred plants.

63. The method of claim 52 wherein the marker gene is deleted at a frequency of at least 0.1%.

64. The method of claim 52 wherein the marker gene is delcted at a frequency of at least 0.6%.

65. The method of claim 52 wherein the marker gene is deleted at a frequency of at least 2%.